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PAPER

Fungal DNA in hotel rooms in Europe and Asia—associations with latitude, precipitation, building data, room characteristics and hotel ranking

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There is little information on the indoor environment in hotels. Analysis of fungal DNA by quantitative PCR (qPCR) is a new method which can detect general and specific sequences. Dust was collected through swab sampling of door frames in 69 hotel rooms in 20 countries in Europe and Asia (2007–2009). Five sequences were detected by qPCR: total fungal DNA, *Aspergillus* and *Penicillium* DNA (*Asp*/*Pen* DNA), *Aspergillus versicolor* (*A. versicolor* DNA), *Stachybotrys chartarum* (*S. chartarum* DNA) and *Streptomyces* spp. (*Streptomyces* DNA). Associations were analysed by multiple linear regression. Total fungal DNA (GM = 1.08×10^8 cell equivalents m^{-2}; GSD = 6.36) and *Asp*/*Pen* DNA (GM = 1.79×10^7 cell equivalents m^{-2}; GSD = 10.12) were detected in all rooms. *A. versicolor* DNA, *S. chartarum* DNA and *Streptomyces* DNA were detected in 84%, 28% and 47% of the samples. In total, 20% of the rooms had observed dampness/mould, and 30% had odour. Low latitude (range 1.5–64.2 degrees) was a predictor of *Asp*/*Pen* DNA. Seaside location, lack of mechanical ventilation, and dampness or mould were other predictors of total fungal DNA and *Asp*/*Pen* DNA. Hotel ranking (Trip Advisor) or self-rated quality of the interior of the hotel room was a predictor of total fungal DNA, *A. versicolor* DNA and *Streptomyces* DNA. Odour was a predictor of *S. chartarum* DNA. In conclusion, fungal DNA in swab samples from hotel rooms was related to latitude, seaside location, ventilation, visible dampness and indoor mould growth. Hotels in tropical areas may have 10–100 times higher levels of common moulds such as *Aspergillus* and *Penicillium* species, as compared to a temperate climate zone.

1. Introduction

Hotel buildings are important indoor environments for travellers and hotel staff but there are few studies on the hotel indoor environment. The only health studies available deal with ocular and respiratory symptoms in hotel indoor water parks[1,2] or indoor swimming pools,[3,4] symptoms possibly related to the chlorination. The environmental studies in hotels to date have been dealt with radon in hot spring hotels,[5] environmental tobacco smoke exposure,[6,7] polybrominated flame retardants[8] and mite and pet allergens.[9] One article described severe problems with mould growth during construction of a hotel.[10] Finally one study described an integrated indoor air quality audit in a Taiwanese hotel. The main problems involved too low room temperature, insufficient ventilation, formaldehyde contamination and high levels of airborne viable bacteria.[11] The concern about microbial exposure in hotels has mainly been on outbreaks of infectious diseases, such as Legionella infection,[12–14] norovirus

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Environmental impact

Indoor microbial contamination can influence the risk for asthma and allergy, and there is a need for simple methods to monitor indoor levels of moulds. This paper has applied a simple monitoring method for moulds in hotel in different parts of Europe and Asia, and generated new information about determinants of indoor mould levels in hotel rooms. Seaside location, low latitude, lack of mechanical ventilation, observed dampness of moulds, odour, and the quality of the interior of the hotel room were important predictors of mould levels measured as fungal DNA. Dust sampling by cotton swab and quantitative PCR can be a sensitive and convenient method to monitor indoor fungal concentrations. The lack of data on the indoor environment in hotels illustrates the need for further hotel studies.

infections[15,16] and cryptosporidiosis.[17] Apart from these case reports, we found no studies on mould contamination in hotels.

There is an increased concern about possible health effects of indoor dampness and mould.[18] Detection and quantification of indoor fungi are now possible by using quantitative polymerase chain reaction (qPCR, sometimes called real time PCR).[19] The qPCR method offers a new venue for rapid detection and quantification of fungi in indoor samples.[19–21] We have previously applied detection of fungal DNA by qPCR in swab samples from door frames as a quick and convenient method to monitor mould contamination in Swedish day care centres.[22,23]

The aim was to measure levels of fungal DNA in swab samples from the hotel room in Europe and Asia and to relate them to climate conditions, location, building and room characteristics, perceived indoor air quality and ranking of the hotel. Five DNA sequences were analysed. Two were aimed at detecting a large number of fungal species that could occur in the indoor environment. Three were more specific sequences aimed at detecting two particular fungi producing mycotoxins (*Aspergillus versicolor*, *Stachybotrys chartarum*), as well as *Streptomyces* sp. The latter is a large genus of Gram-positive bacteria and some species can produce inflammatory reactions[24] and geosmin, a fungal compound with a strong odour.[25]

2. Materials and methods

2.1. Selection of population

Swab samples were taken from all hotel rooms in 69 hotels visited in 20 countries by an occupational hygienist during 18 months (October 2007–May 2009) in connection with business or leisure travelling in Europe and Asia. Data on location, building and room characteristics, perceived indoor air quality and ranking of the hotel were collected. One room was included in each hotel.

2.2. Assessment of location and building data

Data on latitude and longitude of the hotel location and annual precipitation were collected from various Internet sites. The star rating of the hotel was taken from the homepage of the hotel or booking agency. Data on independent ranking were available from Trip Advisor (www.tripadvisor.com) for 52 hotels. This ranking includes an overall score of 0–5 in steps of 0.5 made by the Trip Advisor company itself, weighing hotel information with reviews by visitors. A score based on visitors' ratings only is also offered, giving each hotel a ranking among all hotels reviewed in the same location. The floor level of the investigated hotel room was noted by the occupational hygienist at check in. Through inspection, the size of the hotel, type of hotel (apartment *vs.* room type) and closeness to the seaside were determined. The type of ventilation, central air conditioning, local air conditioning unit in the wall, fan in ceiling and type of floor material were noted in the investigated room. Visible mould growth, damp spots, and mouldy stuffy or chemical odour and the presence of cockroaches were noted in the room, as well as in the interior of the hotel. Moreover the quality of the interior of the hotel room was judged by inspection at three levels (very fresh/new, normal standard, old/worn).

2.3. Dust collection

The dust samples for fungal DNA were collected by swabbing a 60 cm^2 surface (1 × 60 cm) on the upper half of the door frame of the door to the main entrance of each hotel room. Each swab was rotated slowly and moved 3 times back and forth over the surface. Two samples were collected by dividing the door frame into a left and a right side, of which the left was sent for fungal DNA analysis. The other swab sample was stored for future analysis.

2.4. Analysis of fungal DNA by qPCR

The method has been previously described in detail.[26] Briefly the cotton swabs were cut into a 2 ml tube and diluted with 120 μl digestion buffer and 5 μl Zymolysate (Yeastar kit, Zymo Research, Orange, CA, USA). Five multiplex reactions were performed in five separate tubes targeting the DNA of the following species: total fungi, *Aspergillus* spp. and *Penicillium* spp. (*Asp*/*Pen*), *Aspergillus versicolor*, *Stachybotrys chartarum* and *Streptomyces* sp. The reaction targeting *A. versicolor* simultaneously amplified an internal positive control that was used to detect PCR inhibition. There was no inhibition detected in any sample. The oligonucleotides used for amplification and detection were designed using the design software Primer Express 2.0 (Applied Biosystems, Foster City, CA, USA). Design was performed by Dynamic Code (Linköping, Sweden) on highly conserved rDNA sequences obtained from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Amplification and detection were performed on a 7300 Real-time PCR Instrument (Applied Biosystems, Foster City, CA, USA) using the Taqman® Universal Master Mix (Applied Biosystems, Foster City, CA, USA). The result was obtained as copies per sample but was expressed as cell equivalents (CE) for each target mould or mould group, assuming one copy per cell. The final result was presented as CE m^{-2} of the swabbed area.

The total fungal DNA sequence was common for 7 *Acremonium* sp., 61 *Alternaria* sp. (including *A. alternata*), 86 *Aspergillus* sp. (including *A. fumigatus* but not *A. versicolor*), *Aureobasidium mansonii*, *Aureobasidium pullulans*, *Cerebella andropogonis*, 38 *Cladosporium* sp. (including *S. herbarium*), 14 *Curvularia* sp., *Cylindrocarpon lichenicola*, 3 *Davidiella* sp., *Epicoccum nigrum*, 27 *Eupenicillium* sp., 6 *Eurotium* sp., 8 *Fusarium* sp., *Hemicarpenteles paradoxus*, *Mycosphaerella macrospora*, *Mycosphaerella tassiana*, *Nectria haematococca*, 17 *Neosartorya* sp., 15 *Paecillomyces* sp., 157 *Penicillium* sp., 3 *Petromyces* sp., *Ramichloridium mackenziei*, 9 *Rhinocladiella* sp., *Sclerocleista ornata*, 12 *Stachybotrys* sp., 3 *Thermoascus* sp. and 48 *Trichoderma* sp. We have used the term "total fungal DNA" for this sequence as in previous publications[26] since it covers a wide range of indoor fungi, mainly *Ascomycetes*, but it does not cover all indoor fungi.

The *Asp*/*Pen* DNA sequence was common for 37 *Aspergillus* sp. (including *A. fumigatus* but not *A. versicolor*), *Davidiella tassiana*, 14 *Eupenicillium* sp., 15 *Eurotium* sp., *Hemicarpenteles paradoxus*, 7 *Neosartorya* sp., *Paecilomyces variotii*, *Paracoccidioides cerebriformis*, 62 *Penicillium* sp. and *Thermoascus aurantiacus*. The *A. versicolor* DNA sequence was specific for *A. versicolor* only (13 GenBank accessions). The *S. chartarum* DNA

sequence was specific for *S. chartarum* (14 accessions) and *S. chlorohalonata* (6 accessions). The *Streptomyces* DNA sequence was common for 187 *Streptomyces* sp., as well as *Micromonospora megalomicea*. Complete files with GenBank accessions numbers can be obtained from the main author.

2.5. Statistical methods

Statistical calculations were performed using the Statistical Package for Social Science (SPSS 17.0). Since fungal DNA is not normally distributed, bivariate analysis was performed using the Mann–Whitney *U*-test, Fisher's exact test, the Kruskal–Wallis test and Kendall's tau beta rank correlation. Multivariate analysis was performed by multiple linear regression, using log transformed data for fungal DNA. For fungal DNA data below the detection limit (< 100 CE m^{-2}), a value of half the detection limit (50) was used in the regression models. Beta values with 95% confidence intervals (CI) were calculated. Taking the antilog values, multiplicative factors with 95% CI were obtained. As an example a multiplicative factor of 5 means that the level of fungal DNA is 5 times higher if the building factor is present as compared to levels when the factor is absent. The observed interior quality in the hotel rooms was coded 1 = very new, 2 = normal standard, 3 = old/worn. Using the data available from Trip Advisor, a visitors ranking index (range 0–100%) with 100% as the highest rank was calculated by the authors by dividing the rank of the specific hotel by the number of ranked hotels, and subtracting this ratio from 1, and multiplying by 100 to get % values.

3. Results

3.1. Countries, latitude and climate

A total of 72 hotels from 20 countries were visited from October 2007–May 2009. Hotel data were missing for 2 hotels and swab samples were missing from one hotel. The remaining 69 hotels were included in the study (one room per hotel). In Asia, the hotels ($N = 43$) were located in China/Taiwan ($N = 11$), Malaysia ($N = 9$), Thailand ($N = 9$), Vietnam ($N = 7$), Japan ($N = 5$), Cambodia ($N = 1$) and Iran ($N = 1$). In Europe, the hotels ($N = 26$) were located in Spain ($N = 5$), Italy ($N = 4$), Sweden ($N = 3$), France ($N = 3$), Portugal ($N = 2$), United Kingdom ($N = 2$), Norway ($N = 2$), Denmark ($N = 1$), Germany ($N = 1$), Poland ($N = 1$), Estonia ($N = 1$) and Iceland ($N = 1$). Since it was not meaningful to analyse differences between countries, annual precipitation and latitude were used as climatological and geographical variables. Latitude and precipitation differed between Asia and Europe. Mean latitude was 16 degrees in Asia (range 1–40) and 48 degrees in Europe (range 36–64), while mean annual precipitation was 2080 mm in Asia (range 400–3200) and 740 mm in Europe (range 240–2250). In Asia, only two hotels were at a location with less than 1000 mm precipitation per year (Beijing and Teheran). In Europe only one hotel was at a location with more than 1000 mm precipitation per year (Bergen). In tropical and subtropical areas, there is a dry season and a rainy season. Most sampling in Asia was performed during the dry season.

3.2. Location and building data

All hotels with seaside location (<100 m) were situated in Asia (21% *vs.* 0%, $p = 0.01$). Three of the hotel rooms in Asia (7%) had cockroach, none in Europe. Observed dampness or mould growth was more common in Asian hotel rooms as compared to European (28% *vs.* 7%; $p = 0.04$). Hotel rooms at 20 degrees latitude or below had 42% dampness/mould, while those at 20 to 40 degrees had 9% dampness/mould and those at latitudes above 40 degrees only 5% dampness/mould ($p = 0.002$). Data on location, hotel characteristics and observed room characteristics are given in Tables 1 and 2. There were wall-to-wall carpets in 45% of the rooms, 30% had stone floor material, 19% had wooden floor and 6% had plastic material.

3.3. Fungal DNA levels

Total fungal DNA and *Asp*/*Pen* DNA were detected in all swab samples. *A. versicolor* DNA, *S. chartarum* DNA and *Streptomyces* DNA were detected in 84%, 28% and 47% of all samples, respectively. Geometric mean was 1.08×10^8 CE m^{-2} (GSD = 6.36) for total fungal DNA, 1.79×10^7 CE m^{-2} (GSD = 10.12) for *Asp*/*Pen* DNA and 5.10×10^4 CE m^{-2} (GSD = 6.33) for *A. versicolor* DNA. The arithmetic mean concentration was 5.42×10^5 CE m^{-2} (range <100 to 1.54×10^7) for *A. versicolor* DNA, 2.03×10^3 CE m^{-2} (range <100 to 19 800) for *S. chartarum* DNA and 7010 CE m^{-2} (range <100 to 124 000) for *Streptomyces* DNA.

3.4. Associations between fungal DNA level, location and building data

As a first step, bivariate analysis was performed for total fungal DNA and *Asp*/*Pen* DNA, since these were found at the highest levels. Hotels in Asia had higher levels of *Asp*/*Pen* DNA than European hotels and the level of total fungal DNA decreased with higher star ranking. Moreover, larger hotels had lower levels of total fungal DNA. Total fungal DNA and *Asp*/*Pen* DNA were higher in hotels with rural location, as compared to

Table 1 Hotel characteristics for 69 hotels in 20 countries

Hotel characteristics/location		*N*	Percent (%)
Latitude northern hemisphere	0–20	26	38
	21–40	24	35
	>40	19	27
Continent	Europe	26	38
	Asia	43	62
Star rating	≤2	22	32
	3	14	20
	4	21	30
	5	12	18
Location	Rural area	12	17
	Suburban area	10	14
	Town/city	24	36
	Mega city	23	33
Size of the hotel	Small (<10 rooms)	11	16
	Medium (10–100 rooms)	37	54
	Large (>100 rooms)	21	30
Seaside location	No	47	68
	100–500 metres	13	18
	<100 metres	9	4

Table 2 Room characteristics for 69 hotel rooms in 20 countries

Room characteristics		*N*	Percent (%)
Type of ventilation	Natural ventilation only	30	44
	Mechanical ventilation	39	66
Air conditioning unit (A/C) in the wall	No	50	73
	Yes	19	27
Wall-to-wall carpet	No	38	55
	Yes	31	45
Dampness or mould	No	55	80
	Yes[a]	14	20
Any odour	No odour	48	70
	Any type of odour[b]	21	30
Rating of interior/furniture	Old/worn	15	22
	Medium	19	27
	High/new	35	61

[a] Visible indoor mould growth in 8 rooms and damp spots/sign of leakage in 7 rooms. [b] Mouldy odour in 7 rooms, stuffy odour in 11 rooms and chemical odour in 3 rooms.

hotels in suburban or urban areas or mega cities. Hotels close to the seaside (<100 m) had higher levels of total fungal DNA and *Asp*/*Pen* DNA (Table 3). There were decreasing levels of total fungal DNA and *Asp*/*Pen* DNA with latitude, and increasing levels of *Asp*/*Pen* DNA at higher annual precipitation (Table 4).

Ranking of the hotel from the Trip Advisor site was available for 52 hotels. Hotels with a higher overall ranking had lower levels of total fungal DNA. Similar results were obtained for the ranking based on the rank order among hotels at the location where the hotel was located. In addition, total fungal DNA and *Asp*/*Pen* DNA were lower if the room was located at a higher floor level in the hotel (Table 4).

3.5. Associations between fungal DNA level and room characteristics

As a next step, associations were analysed for observed room characteristics. When classifying the floor level in four classes, the highest levels of total fungal DNA were found in rooms at ground level or the top floor. Hotel rooms with a mechanical ventilation system of any type had lower levels of total fungal DNA and *Asp*/*Pen* DNA, and rooms with a local air conditioning unit (local A/C) in the wall had higher levels of *Asp*/*Pen* DNA. There was no difference in fungal DNA levels between stone floors and wooden floors (data not shown), but rooms with wall-to-wall carpeting had lower levels of total fungal DNA and *Asp*/*Pen* DNA ($p = 0.02$). Rooms with any observed sign of dampness or mould growth had higher levels of total fungal DNA and *Asp*/*Pen* DNA. The location of the signs of dampness or mould growth was mostly at the ceiling or in the bathroom, and in no case near the door frame where we collected the samples. Finally the subjective rating of the quality of the interior (textiles, walls, furnitures) of the hotel rooms (new/fresh, normal, old/worn) was negatively related to total fungal DNA and *Asp*/*Pen* DNA, which means lower levels of fungal DNA if the quality was better (Table 5). In addition, there was a strong association between ranking of the interior and the presence of dampness or mould, even if this observation was not included in the ranking. Rooms ranked as new/fresh had only 3% dampness/mould, rooms ranked as normal standard had 25%, and rooms ranked as old/worn had 53% dampness or mould ($p < 0.001$).

3.6. Associations between fungal DNA level, location, building and room characteristics by multivariate analysis

In total, 12 factors related to location, building or room characteristics were significantly related to either total fungal DNA, or *Asp*/*Pen* DNA, or both (not including the 4 ranking variables). Log transformed data for total fungal DNA and *Asp*/*Pen* DNA were analysed by forward stepwise linear multiple regression analysis (inclusion level $p = 0.05$). A total of five variables (latitude, seaside location, floor level, mechanical ventilation and observed dampness or mould) remained significant for total fungal DNA or *Asp*/*Pen* DNA. The floor level was omitted from further analysis since it was positively related to mechanical ventilation ($p = 0.001$) and hotel size and mechanical ventilation

Table 3 Associations between building location, building characteristics and fungal DNA ($N = 69$)[a]

			Total fungal DNA			*Asp*/*Pen* DNA		
Building location/characteristics		*N*	GM[b]	GSD	*p*-Value	GM[c]	GSD	*p*-Value
Continent[d]	Europe	26	0.87	2.87	0.78	0.51	3.35	0.001
	Asia	43	1.31	8.85		3.98	12.1	
Number of stars	<3	22	2.13	4.35	0.04	3.58	9.95	0.45
	3	14	1.56	9.05		2.36	16.1	
	4	21	0.62	2.91		0.89	5.61	
	5	12	0.66	11.0		1.43	13.1	
Size of the hotel	Small	11	4.17	6.27	0.02	11.69	15.5	0.07
	Medium	37	1.01	4.78		1.17	6.91	
	Large	21	0.68	7.75		1.53	10.5	
Location	Rural	12	5.45	7.32	0.03	17.93	13.2	0.02
	Suburban	10	0.67	5.30		0.84	4.22	
	Urban	24	0.95	4.71		1.41	9.12	
	Mega city	23	0.73	5.84		1.04	7.04	
Seaside location	No	47	0.81	5.77	0.002	1.13	8.65	0.001
	100–500 m	13	0.80	4.21		1.27	5.13	
	<100 m	9	9.73	4.04		38.55	5.58	

[a] GM: geometric mean and GSD: geometric standard deviation. [b] $\times 10^8$ CE m^{-2}. [c] $\times 10^7$ CE m^{-2}. [d] Analyzed by the Mann–Whitney *U*-test, the rest of the variables analyzed by the Kruskal–Wallis test.

Table 4 Correlation between fungal DNA and climate zone, building age and ranking of the hotels ($N = 69$)

Building location/characteristics		Total fungal DNA		*Asp*/*Pen* DNA	
Variables	Range	Tau beta	*p*-Value[a]	Tau beta	*p*-Value[a]
Latitude	1.5–64.2 degrees	−0.17	0.04	−0.40	<0.001
Annual precipitation	240–3200 mm	0.11	0.20	0.36	<0.001
Building age	1–259 years	0.06	0.48	−0.07	0.39
Overall ranking	2.5–4.5	−0.27	0.01	−0.12	0.25
Rank order at location	0–100%	−0.31	0.001	−0.08	0.43

[a] Kendall's tau beta rank test.

is a more plausible explanation to reduced levels of fungal DNA than the floor level as such. The remaining four variables were entered simultaneously into two multiple linear regression models. Multiplicative factors with 95% CI were calculated from the beta-values. For comparison, data from unadjusted models for the same variables were added (Table 6). All associations except between latitude and total fungal DNA remained significant in the adjusted models. For latitude, total fungal DNA was significant in the bivariate analysis but not after adjusting for seaside location, mechanical ventilation and observed dampness or mould. This could be due to the fact that the latitude was lower for hotels with the seaside location ($p < 0.001$) and observed dampness and mould ($p = 0.01$). If moving from 50 degrees north to the equator (*e.g.* from London to Singapore), the levels of total fungal DNA would increase 1.4 times by the adjusted model and 5.6 times by the unadjusted model. The level of *Asp*/*Pen* DNA would increase 10.0 times by the adjusted model and 50.1 times by the unadjusted model (Table 6). Rooms in hotels situated less than 100 metres from the shore would have 5.1 times higher levels of fungal DNA in the adjusted model and 5.2 times higher levels of *Asp*/*Pen* DNA. Rooms without a mechanical ventilation system would have 2.3 times higher levels of fungal DNA in the adjusted model and 2.2 times higher levels of *Asp*/*Pen* DNA. Rooms with dampness or mould growth would have 5.9 times higher levels of fungal DNA and 8.9 times higher levels of *Asp*/*Pen* DNA (adjusted models). The explanatory values (*R*-square) for the models with the four dependent variables were 44% for total fungal DNA and 63% for *Asp*/*Pen* DNA.

3.7. Associations between fungal DNA level and ranking variables

As a next step, we analysed associations between fungal DNA and the four ranking variables: the number of stars, overall rank by Trip Advisor, rank order at location by Trip Advisor, and the observer's own rating of the quality of the interior of the room. We analysed the ranking variables in separate models since it could be expected that the ranking would be influenced by the previous guests observation of the room and hotel characteristics. The rank order at location ($p = 0.02$) and the observer's own rating of the interior ($p < 0.001$) were both predictors for total fungal DNA. Multiplicative factors with 95% CI were calculated for adjusted models with the two significant rankings. There were 9.0 times higher levels of total fungal DNA (95% CI 3.0–27) in a room rated as old/worn than in one rated fresh/new, and 7.8 times higher levels of total fungal DNA (95% CI 1.3–48) in a room in a hotel ranked lowest than one ranked highest among all hotels at the specific location. For *Asp*/*Pen* DNA, there proved to be 13.1 times higher levels of *Asp*/*Pen* DNA (95% CI 2.9–60) in a room rated as old/worn than in one rated fresh/new. The explanatory values (*R*-square) for the ranking models were 38% for total fungal DNA and 24% for the *Asp*/*Pen* DNA model (two dependent variables).

Table 5 Associations between room characteristics and fungal DNA ($N = 69$)[a]

		Total fungal DNA				*Asp*/*Pen* DNA			
Room characteristics		*N*	GM[b]	GSD	*p*-Value	*N*	GM[c]	GSD	*p*-Value
Floor level[d]	Ground = 1	13	3.62	6.98	0.006	13	6.29	12.3	0.08
	2–4	22	1.11	4.51		22	1.51	8.19	
	5–22	20	0.40	4.54		20	0.75	5.54	
	Top floor	14	1.66	7.50		14	2.87	16.5	
Mechanical ventilation	No	30	2.23	5.24	0.001	31	3.66	10.2	0.02
	Yes	39	0.61	6.12		39	1.01	8.77	
A/C in the wall	No	50	0.92	5.59	0.15	50	1.23	8.67	0.01
	Yes	19	1.23	8.67		19	5.23	11.6	
Wall-to-wall carpets	No	38	2.02	5.97	0.002	38	3.19	12.0	0.02
	Yes	31	0.54	5.26		31	0.93	6.87	
Dampness or mould	No	55	0.72	4.68	<0.001	55	0.93	5.81	<0.001
	Yes	14	6.52	6.74		14	26.2	10.9	
Odour	No	48	1.07	5.77	0.61	48	1.59	10.1	0.43
	Yes	21	1.25	7.91		21	2.56	10.6	
Quality of interior[d]	High/new	35	0.77	4.53	<0.001	35	0.81	5.28	0.002
	Normal	19	1.30	4.35		19	1.56	8.17	
	Old/worn	15	7.14	9.21		15	1.54	14.5	

[a] GM: geometric mean; GSD: geometric standard deviation; and A/C = air conditioning. [b] $\times 10^8$ CE m^{-2}. [c] $\times 10^7$ CE m^{-2}. [d] Analyzed by the Kruskal–Wallis test, the rest of the variables analyzed by the Mann–Whitney *U*-test.

Table 6 Multiple linear regression analysis for total fungal DNA and *Asp/Pen* DNA ($N = 69$)[a]

	Log total fungal DNA		Log *Asp/Pen* DNA		Total fungal DNA		*Asp/Pen* DNA	
Factors	U-β (95% CI)	A-β (95% CI)	U-β (95% CI)	A-β (95% CI)	U-*f* (95% CI)	A-*f* (95% CI)	U-*f* (95% CI)	A-*f* (95% CI)
Latitude (per 10 degree)	−0.15 (−0.25, −0.05)	−0.03 (−0.13, 0.07)	−0.34 (−0.44, −0.24)	−0.20 (−0.30, −0.10)	5.6 (1.8–17.8)[b]	1.4 (0.4–4.5)[b]	50.1 (15.8–159)[b]	10.0 (3.2–31.6)[b]
Seaside location (<100 metre)	0.72 (0.32, 1.13)	0.71 (0.17, 1.24)	1.02 (0.52, 1.51)	0.71 (0.16, 1.27)	5.3 (2.1–13.4)	5.1 (1.5–14.1)	10.4 (3.3–39.7)	5.2 (1.4–18.4)
Mechanical ventilation	−0.61 (−0.97, −0.25)	−0.35 (−0.69, −0.02)	−0.60 (−1.06, −0.13)	−0.34 (−0.68, 0.002)	4.1 (1.8–9.3)	2.3 (1.0–4.9)	4.0 (1.3–11.7)	2.2 (1.0–4.8)
Dampness or mould	0.96 (0.54, 1.38)	0.77 (0.36, 1.18)	1.45 (0.96, 1.94)	0.95 (0.53, 1.37)	9.1 (3.5–23.9)	5.9 (2.3–15.1)	28.1 (9.0–87.5)	8.9 (3.4–23.2)

[a] U-β: unadjusted beta value; A-β: adjusted beta value; U-*f*: unadjusted multiplicative factor; and A-*f*: adjusted multiplicative factor. [b] Calculated for a change of latitude of 50 degrees.

3.8. Associations for species-specific fungal DNA

Finally we performed crude bivariate analysis for species-specific fungal DNA. *A. versicolor* DNA levels were lower in rooms with mechanical ventilation ($p = 0.005$) and higher in rooms with signs of dampness or moulds ($p = 0.03$) and negatively related to the ranking of the interior of the room. *S. chartarum* DNA was higher in rooms with any odour ($p = 0.01$) and lower at seaside location (<100 m) ($p = 0.045$). *Streptomyces* DNA levels were lower in rooms with mechanical ventilation ($p = 0.02$), and negatively correlated with star ranking (Kendal tau beta −0.27; $p = 0.007$), the size of the hotel as 4 categories (Kendal tau beta −0.28; $p = 0.007$), and the floor level of the room as 4 categories (Kendal tau beta −0.24; $p = 0.02$), and the ranking of the interior of the room as 3 categories (Kendal tau beta −0.23; $p = 0.007$). There were no associations between any species-specific fungal DNA and continent, latitude, annual precipitation, rural or urban location (4 categories), building age, wall-to wall carpet, A/C in the wall, overall ranking or rank order at the location by Trip Advisor.

The nine significant variables were kept simultaneously in three multiple linear regression models, using log transformation of *A. versicolor* DNA, *S. chartarum* and *Streptomyces* DNA data. The only significant association was for ranking of the interior of the room and *A. versicolor* DNA (adjusted beta 0.33; 95% CI −0.10 to 0.56; $p = 0.005$), and for any odour and *S. chartarum* DNA (adjusted beta 0.55; 95% CI 0.15 to 0.95; $p = 0.008$). For *Streptomyces* DNA there were associations for ranking of the interior of the room (adjusted beta 0.36; 95% CI 0.09 to 0.62; $p = 0.008$) as well as the floor level of the room as 4 categories (adjusted beta −0.27; 95% CI −0.48 to −0.07; $p = 0.01$). The explanatory values (*R*-square) for the three models were 11% for *A. versicolor* DNA, 10% for *S. chartarum* and 20% for *Streptomyces* DNA.

3.9. Effects of flooding on the fungal DNA level

Two of the hotels had been exposed to severe flooding during the tsunami in Thailand in 2004 but were refurbished. They had the highest levels of total fungal DNA (4.6–8.2 $\times$ 10^9 CE m^{-2}) and *Asp/Pen* DNA (3.9–6.3 $\times$ 10^9 CE m^{-2}) but were no outliers statistically. The levels of *A. versicolor* DNA or *S. chartarum* DNA were below the detection limit in both hotels (<100 CE m^{-2}). One had the highest level of *Streptomyces* DNA (1.2 $\times$ 10^6 CE m^{-2}) and the other had no *Streptomyces* DNA (<100 CE m^{-2}). Re-running the final regression models for climate/building factors and hotel rating, excluding these two hotels, did not change any of the regression coefficients or the statistical significance.

3.10. Associations for *Asp/Pen* DNA and the total fungal DNA ratio

Finally the climatological associations were studied for the ratio between *Asp/Pen* DNA and total fungal DNA. The ratio would be independent of cleaning habits. In the crude analysis, the ratio was higher at lower latitude (Kendall's tau-beta −0.45; $p < 0.001$), higher at higher annual precipitation (Kendall's tau-beta 0.42; $p < 0.001$) and higher in Asia as compared to Europe. The mean ratio was 0.46 (SD = 0.30) in Asia, and 0.11 (SD = 0.16) in

Europe ($p < 0.001$). At latitudes 0–20 the mean ratio was 0.53 (SD = 0.31), at latitudes 21–40 it was 0.30 (SD = 0.26) and at >40 it was 0.11 (SD = 0.17). When applying multiple linear regression analysis, including latitude, precipitation, and continent, the difference between Asia and Europe was no longer significant ($p = 0.54$), while latitude and annual precipitation remained significant. In this model, moving from north 50 degrees to the equator (*e.g.* from London to Singapore) would increase the ratio by 0.29 (95% CI 0.05–0.54), and increasing the annual precipitation by 1000 mm per year would increase the ratio by 0.11 (95% CI 0.01–0.22). The explanatory value (*R*-square) for the model was 38%.

4. Discussion

Fungal DNA could be detected in all hotel rooms, but levels varied by an order of magnitude of 10^3 to 10^4. We found that low latitude, seaside location, lack of a mechanical ventilation system and observed dampness and mould growth were predictors of fungal DNA. Moreover hotel ranking at a major independent hotel ranking site and observed quality of the interior were related to fungal DNA. The explanatory values of the multiple regression models (*R*-square) were relatively high (44–63%) for total fungal DNA and *Asp*/*Pen* DNA but low (10–20%) for species-specific DNA. To our knowledge this is the first study on mould contamination in hotels in different climate zones.

The study was conducted in one room in each hotel visited by the main author over an 18 month period, with the exception of three hotels (4%) with missing data. The study was blinded and the hotels were located in 20 countries in Asia and Europe and covered a wide range of climate zones and types of hotels. One limitation was that we did not include hotels from very dry climate zones where fungal DNA levels could have been lower and that we did not include monthly temperature or monthly precipitation in our models. Moreover since most of the samples in Asia were collected during the dry season, the effect of latitude may have been underestimated. For the main part the associations were highly significant ($p < 0.01$ or $p < 0.001$) and consistent between bivariate and multivariate analyses. Thus, we do not think that our conclusions are seriously influenced by recall bias or selection bias.

Analysis of microbial components in vacuumed settled dust has been used as a proxy for exposure in many studies but analysis of fungal DNA in swab samples is a new method for monitoring surface contamination.[22,23,26] A sample can be collected easily but the amount of dust is small and dust cannot be weighed. Hotel rooms are usually cleaned on a daily basis, but since door frames in hotels are rarely cleaned, the settled dust could reflect an integrated value of airborne fungal DNA settling during a longer period (several months). The disadvantage of this could be that the sample has been affected by local conditions in the wall near the door frame, and may not be representative of the indoor environment in other parts of the room.

Apart from the issue of odour in the hotel room, our study did not include any data on health or indoor air quality complaints. There are some epidemiological studies available using qPCR to detect fungal exposure. One study found that asthmatic children had higher concentration of fungal DNA from two species in bedroom dust, as compared to controls, while fungal DNA from seven other species was found in lower concentration.[27] In another study, allergic patients had higher concentration of *Aspergillus versicolor* DNA at home as compared to matched controls.[28] In another home environment study, a Relative Moldiness Index (RMI) was developed, and was found to be associated with respiratory illness in the children.[21] Thus it seems that fungal DNA measured by qPCR can be valuable in epidemiological studies. Varying extraction efficiency for different species, or for different extraction methods, is a potential weakness of qPCR. The need of optimizing the extraction method for fungal DNA has been demonstrated for specific primers,[29] but the magnitude of this problem is not known for universal primers. Different types of extraction methods are available: we used enzymatic degradation by YeaStar. Validation studies are needed that compare universal primers with other methods for total moulds such as β-1,3-glucane,[30] ergosterol,[31] or the CAMNEA method.[32] We had a pragmatic approach and applied universal and species-specific qPCR in a field study, and found expected and reasonable association with building characteristics mainly for total fungal DNA and *Asp*/*Pen* DNA.

We found relatively high levels of fungal DNA, geometric mean (GM) was 1.08×10^8 CE m^{-2} for total fungal DNA and 1.79×10^7 CE m^{-2} for *Asp*/*Pen* DNA. Species-specific sequences were common, including *S. chartarum* DNA, but levels were much lower. In a previous study from Swedish day care centres, GM was 4.2×10^6 CE m^{-2} for total fungal DNA and 8.4×10^3 CE m^{-2} for *Asp*/*Pen* DNA.[23] In another study from schools in a tropical area (Johor Bahru, Malaysia),[26] GM was 5.7×10^8 CE m^{-2} for total fungal DNA and 5.0×10^7 CE m^{-2} for *Asp*/*Pen* DNA.[26] These differences could be due to the climate zone effect.

We found higher levels of *Asp*/*Pen* DNA and a higher ratio between *Asp*/*Pen* DNA and total fungal DNA at lower latitude in the final regression models. Moreover the *Asp*/*Pen*/total fungal DNA ratio was higher at higher annual precipitation, when adjusting for continent and latitude. The source of the indoor fungal DNA could be from both the indoor and the outdoor environment. One study measured simultaneously airborne levels of fungal DNA from 36 mold species by qPCR. Only three species (*Aspergillus penicillioides*, *Cladosporium cladosporoide* types 1 and 2 and *Cladosporium herbarum*) had a correlation between the concentration in indoor and outdoor samples, suggesting that indoor and outdoor environments may have different fungal compositions.[33] There are few other comparative studies on levels of indoor moulds at different climate zones. One large study on viable indoor and outdoor moulds in different parts of the USA found a latitude effect, with the lowest indoor levels in the Northeast and the lowest outdoor levels in the Northwest.[34] Another global study on indoor fungal contamination, using both taxonomic and phylogeny-informative molecular markers by PCR, found that the fungal diversity was higher in temperate climate zones than in the tropics. Latitude was the best predictor of diversity, while the building function had no effect on the indoor fungal composition.[35] If we consider the *Asp*/*Pen*/total fungal DNA ratio as a crude measure of diversity, our results lean in the same direction.

Initially, we found higher levels of *Asp*/*Pen* DNA in Asia as compared with Europe but this difference disappeared when

adjusting for latitude and annual precipitation. In tropical and subtropical areas the wet season is expected to facilitate mould growth. One possible reason for the observed effect of seaside location could be that buildings very near the beach would have constantly high air humidity all through the year, facilitating mould growth, and being at risk for flooding as well. All seaside hotels were situated in Asia. When analyzing species specific DNA, we found lower levels of *Streptomyces* DNA, a soil bacteria at the higher floor level of the room. In a previous study from USA it was concluded that indoor *Streptomyces* DNA may have mostly outdoor sources,[24] and thus a lower level could be expected when being more far away from the ground level.

We found that hotel rooms with observed signs of dampness or mould growth had higher levels of total fungal DNA and *Asp*/*Pen* DNA, even when adjusting for other significant factors including latitude. This association is in agreement with the previous Swedish day care centre study, using the same swab method.[22] Moreover, we found an association between odour and levels of *S. chartarum* DNA. The Swedish day care centre study found associations between reported odour and total fungal DNA but not between odour and *S. chartarum* DNA.[22] The location of the dampness was mostly at the ceiling or in the bathroom, and in no case near the door frame where we collected the samples. The odour was mainly perceived as stuffy. A previous study from USA found that the sum of the logarithmic concentration in vacuumed dust of fungal DNA from six species (four *Aspergillus* species, *Eurotium* sp., and *Cladosporium sphaerospermum*) was associated with observed moulds in dwellings.[20] Hotel rooms with a mechanical ventilation system had lower levels of total fungal DNA, *Asp*/*Pen* DNA, and *A. versicolor* DNA, as compared to those with natural ventilation, only. Our results were expected since one function of mechanical ventilation is to remove indoor pollutants including water vapor, and to decrease relative air humidity, and as a consequence reduce the risk of fungal growth. The beneficial effect would be expected to be most pronounced in warmer climate zones with high relative air humidity.

We tested four different hotel ranking systems with respect to fungal DNA levels. The strongest associations were found for a ranking of the quality of the interior of the fabrics and furniture in the hotel room in three levels (new, normal, and worn) by the visitor. We did not have information on the age of the fabrics and furniture. However, it is likely that regular redecoration of hotel rooms will reduce the levels of fungal DNA, since particle pollutants tend to accumulate in older materials.

5. Conclusions

Fungal DNA is common in hotels in both Asia and Europe, and is related to low latitude, seaside location, lack of a mechanical ventilation system, odour, observed dampness and indoor mould growth, as well as the ranking of the hotel and the interior of the hotel room. Hotel rooms in tropical areas could have 10–100 times higher levels of common moulds such as *Aspergillus* and *Penicillium* species, as compared to those in a temperate climate zone. The lack of data in the literature on the indoor environment in hotels illustrates the need for further hotel studies.

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